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6-OHDA Lesions of the Hypothalamus: Interaction of Aphagia, Food Palatability, Set-point for Weight Regulation, and Recovery of Feeding[1]

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MYERS, R. D. AND G. E. MARTIN. *6-OHDA lesions of the hypothalamus: Interaction of aphagia, food palatability, set-point for weight regulation, and recovery of feeding.* PHARMAC. BIOCHEM. BEHAV. 1(3) 329–345, 1973.–6-Hydroxydopamine (6-OHDA) exerted a marked effect on normal eating behavior when injected bilaterally into certain regions of the brainstem of the rat. The drug was administered in a volume ranging from 0.5 to 1.5 μl at sites in the hypothalamus and mesencephalon extending from AP 7.0 to 2.2. In 41 of 42 rats, 6-OHDA did not elicit an ingestive response following its injection in doses of 5, 8, 15, or 30 μg per site. Rather, the immediate effect of the drug was a profound aphagia and adipsia which was either short-lived, chronic, or lethal depending on the anatomical locus of injection and the concentration of the drug given. The crucial factor in the reversal of anorexia and the reinstatement of a normal feeding pattern was the nature of the diet itself. Recovery from a 6-OHDA syndrome was enhanced or attenuated in 28 of 42 rats simply by altering the esculent property of the diet. In a test of the Powley-Keesey hypothesis for weight regulation, 4 of 5 of those rats reduced by food deprivation to 75% of their normal body weight recovered from the highest dose (30 μg) of 6-OHDA, whereas all but one rat died in another high dose group in which body weight had not been previously reduced. However, when 6-OHDA was injected in lower doses into the hypothalamus, the level of body weight did not necessarily determine the time course of recovery nor the extent to which drinking and feeding resumed. At 50 of 57 sites in which 6-OHDA had been injected, 5 μg of norepinephrine failed to elicit any eating response. Neither eating nor drinking was evoked by the micro-injection of acetylcholine-eserine, angiotensin, or dopamine at many of these sites or by calcium (Ca^{++}) injected into the cerebral ventricle. Although these findings give experimental support to the supposition that a catecholaminergic mechanism may be involved in food and water intake, it is clear that the metabolic weight regulation system, gustatory, motor and other factors required for ingestive behavior are all affected to a differential degree by the chemical lesion produced by 6-OHDA.

Palatability Food intake 6-Hydroxydopamine lesion Deprivation Norepinephrine pathway Hypothalamus and food intake Chemical lesion Set-point for body weight

IT SEEMS probable that for the regulation of food intake, norepinephrine serves as a vital link in the neurotransmitter process present in the hypothalamus. This supposition is derived from a combination of pharmacological, anatomical and physiological evidence. First, eating occurs when norepinephrine (NE) is injected into the hypothalamus of the rat [4, 15, 33] or monkey [31,49], or into the cerebral ventricle of a rat that is aphagic after destruction of its lateral hypothalamus [2]. Related to these results is the finding that the prior injection into the hypothalamus of an adrenergic antagonist may block NE-induced feeding [5, 21,33]. Second, NE is stored endogenously in the nerve terminals within the hypothalamus, as shown by the technique of histochemical fluorescence [1,14]. Third, the release of a catecholamine-like substance has been detected in the perfusate collected by means of push-pull cannulae from the hypothalamus of a food-deprived monkey [49]. Further, when this effluent from the hungry donor is perfused similarly within an homologous site in a fully satiated recipient monkey, the latter not only feeds [23] but will press a lever to obtain food [50].

Several questions have been raised about an exclusive role of NE in the hunger circuit. For example, an intraventricular injection of calcium ions (Ca^{++}) causes eating which is not abolished by an alpha-adrenergic antagonist [25]; in addition, the destruction of the nigrostriatal dopamine (DA) pathway also causes the cessation of normal food intake [45]. Finally, Krebs and Bindra [18] have shown that the iontophoretic application of NE or two anorexic agents to cells in the perifornical area and VMH produces a similar effect on firing rates.

A unique method of testing the role of NE in a hunger circuit has evolved with the synthesis of 6-hydroxydopamine (6-OHDA) (2,4,5-trihydroxyphenylethylamine) which, depending on the route of administration, has been shown to deplete endogenous stores of NE at both

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peripheral [19, 27, 41] and central sites [32, 42, 47]. By the relatively specific destruction of catecholamine terminals, a somewhat more selective means of examining the chemically denervated hypothalamus is provided in contrast to that produced by electrolytic lesions.

When cerebral NE is depleted to as low as 19% of the control level, as measured by whole brain assay, following an intraventricular injection of 6-OHDA, no effect on food intake was observed [3, 6, 47]. However, the injection of 6-OHDA directly into the hypothalamus has reportedly produced inconsistent effects on eating behavior. On the one hand, aphagia and adipsia similar to that produced by electrolytic lesions have been observed [34,45]. On the other hand, Evetts *et al.* [13] have reported that the hypothalamic injection of 6-OHDA induces stimulus-bound eating due perhaps to an induced release of NE at the injection site.

In view of the questions raised by earlier investigations, the present study was undertaken to clarify five principal points: first, to determine more precisely the anatomical locus of action of 6-OHDA in attenuating feeding and inducing a lethal aphagia and adipsia; second, to ascertain whether the pharmacological and behavioral sequelae of 6-OHDA could be reversed by altering the constituents and palatability of food offered; third, to find out whether normal ingestive behavior could be reinstated after a 6-OHDA injection; fourth, to test the hypothesis of Powley and Keesey [28] that an animal resets its set-point for regulation of body weight following a chemical lesion to the catecholamine terminals in the hypothalamus; and fifth, to determine whether dopamine, norepinephrine or other endogenous substance could evoke feeding within the very region that had been lesioned previously by 6-OHDA.

METHOD

Animals

Male albino rats of the Sprague-Dawley strain were used which ranged in weight from 380 to 520 g and were 150–190 days of age at the beginning of the experiment. Each animal was housed individually under 12-hr light, 12-hr dark conditions. Water and powdered Wayne Lab Blox were available ad lib to each animal. However, during any predetermined period of food deprivation, the rat was given only that amount of powdered Lab Blox per day which would maintain the animal's body weight in the range of 75–80% of the baseline level.

Surgery

Using methods described previously [24], guide cannulae cut from 22 ga thin wall stainless steel tubing were implanted bilaterally 2 mm below the dura. Hence, the tip rested well above the intended diencephalic site of injection, so that the necrosis of the tissue into which the drug would be given was minimized. The intended site of injection in the rostro-caudal plane of the hypothalamus was calculated using three sets of coordiantes according to the atlas of DeGroot [11]. The guides were set so that injections could be made in: the lateral hypothalamus in 27 animals, AP = 5.4, L = 2.0, HV = −3.3; the perifornical region in 8 animals, AP = 5.8, L = 1.3, HV = 2.0; and the anterior hypothalamus in 6 animals, AP = 6.2, L = 0.8, HV = −2.2. These coordinates were selected since a catecholamine applied within each of these areas evokes a distinct feeding response [4, 10, 33]. After the guide cannulae were lowered to the appropriate position, three stainless steel screws were inserted in the calvarium, and cranioplast cement was packed in and around the cannulae and screws.

Micro-injection Procedures

Each compound was dissolved in an artificial cerebrospinal fluid (CSF), prepared freshly on each day with sterile distilled water in pyrogen-free glassware [24]. The CSF contained Na^+, 127.6mM; K^+, 2.5 mM; Ca^{++}, 1.23 mM; Mg^{++}, 1.0 mM; and Cl^-, 134.5 mM. The drugs used were as follows: dl-norepinephrine (dl-arterenol hydrochloride, Sigma); 3-hydroxytyramine hydrochloride (dopamine,

TABLE 1

DRUGS INJECTED INTRACEREBRALLY AT SITES TREATED WITH 6-OHDA INCLUDING: DOSE (μg), VOLUME OF INJECTION (μl), AND NUMBER OF SITES TREATED

Compound	Dose (μg)	Volume (μl)	Number of Sites
Norepinephrine	0.5	0.5	10
Norepinephrine	5.0	0.5 or 1.5	36
Norepinephrine	10.0	0.5 or 1.5	26
3-Hydroxytyramine	1.0	1.0	10
Acetylcholine-Eserine	2.0	1.5	6
Angiotensin	4.0	1.0	10
Ca^{++}	50.4 mM	10.0*	3

*Injected into the lateral cerebral ventricle.

Sigma); angiotension II (hypertensin II, CIBA); eserine sulphate (Sigma); acetylcholine chloride (Calbiochem). In addition, a solution of Ca^{++} ions 50.4 mM, 40 times in excess of the normal value in extracellular fluid, was added to the artificial CSF. Acetylcholine was mixed with eserine, and the two salts were dissolved in equal proportions. The dose of each compound is expressed in terms of micrograms of the salt. Ascorbic acid, 0.2 mg/ml, was added to the solution of 6-OHDA to prevent oxidation. The dose and volume of the injection used for each drug are listed in Table 1.

The micro-injection cannulae were fashioned from 26 or 28 gauge stainless steel tubing, beveled at the internal end, and connected via PE-20 tubing to a 10 μl Hamilton syringe mounted on a variable speed micro-injection pump. A small air bubble was injected into the PE-20 tubing so that flow of the injection fluid could be monitored constantly during the injection [24]. Immediately before and after each micro-injection, the flow at the tip of the cannula was verified by turning the pump on briefly until a droplet appeared. After the cannula was inserted into the guide tube, the solution was injected into the site over a constant time interval of 45 sec. To permit complete diffusion of the injected solution, the cannula was left in place for 45 sec at the end of each injection [24].

Unless otherwise indicated, each dose of 6-OHDA was given bilaterally within a 15 min interval for each animal. Each of the other drugs was also given bilaterally, but the second injection was not given for one-half hour or longer in the case in which an ingestive response was evoked by the first injection. The solution of excess calcium was injected unilaterally into either one of the lateral ventricles [25]. Each injection was administered to the rat in its home cage, which had been placed on a table two hours prior to the drug administration. For one hour prior to treatment with a drug and for a minimum of one hour after, food and water intakes were recorded and other behaviors were carefully noted. The sequence in which the compounds were administered is indicated under each experiment.

6-OHDA – Dose response and Set-Point for Body Weight

In a group of 27 rats, 13 animals were reduced to 75% of their normal body weight by means of partial food deprivation over a period of 12–18 days. The remaining animals were kept at normal body weight. In each rat 6-OHDA was injected bilaterally at one of three doses: 30 μg/site (high dose); 15 μg/site (medium dose); and 5 μg/site (low dose). The number of animals given each dose and their pre-injection weights together with the volume of injection are presented in Table 2. Volitional food and water intakes as well as body weight were recorded daily for each rat prior to and following the bilateral injections of 6-OHDA.

Because it was necessary to retain each brain for subsequent histological verification of the precise site of injection, the extent of catecholamine depletion was not measured biochemically. However, the doses of 6-OHDA used in the study correspond to those found to deplete endogenous catecholamines when injected similarly into brain tissue [34, 42, 45, 46].

The day following 6-OHDA administration, 5 μg of norepinephrine in 0.5 μl was injected at the identical brain site in 14 nondeprived animals and in 4 deprived animals in an attempt to elicit feeding. The second of the bilateral injections was not made until at least one-half hour after any eating elicited by the first injection had ceased. In addition, NE was injected one day later at a higher, 10 μg, or lower, 0.5 μg dose, at the number of sites indicated in Table 1, for purposes of comparison with the 5 μg injection. Subsequently, several other compounds known to elicit ingestive behavior were applied to the 6-OHDA-treated diencephalic sites. The dose and compounds were: 1 μg of dopamine [26]; 4 μg of angiotensin [12]; and 1 μg of both acetylcholine and eserine mixed in equal proportions [26]. In addition, an artificial CSF solution containing 49.2 mM of calcium in excess of the normal level in extracellular fluid, which produces intense feeding [25], was injected into the lateral ventricle after 6-OHDA administration.

TABLE 2

VOLUME OF INJECTION FOR EACH BILATERAL DOSE OF 6-OHDA GIVEN IN THE DOSE–RESPONSE, SET–POINT EXPERIMENT, INDICATING NUMBER OF ANIMALS TREATED AT EACH BODY WEIGHT

	Dose of 6-OHDA		
Body Weight	High (30 μg)	Medium (15 μg)	Low (5 μg)
Normal	1.5 μl (N = 5)	1.5 μl (N = 5)	0.5 μl (N = 4)
75%	1.5 μl (N = 5)	1.5 μl (N = 4)	0.5 μl (N = 4)

Palatable Food

Two diets were utilized in order to circumvent difficulties involved with tube-feeding aphagic animals. The first was a highly palatable enriched diet (Pa_1) consisting of commercial shortening (Crisco), a powdered therapeutic nutriment (Sustagen), and powdered Wayne lab chow in a proportion of 3:2:5. Animals that would not eat the regular lab chow for the three days following an injection of 6-OHDA were offered this diet. The shortening was liquified by heating and then the other constituents were blended in. A uniform mixture was obtained and solidified upon cooling in a cake pan. Small portions were then cut in cubes. A second diet that was also found to be more palatable to the normal animal than the powdered chow was commercial Wayne Lab Blox in pelletized form (Pa_2).

Injection of 6-OHDA at Sites at Which Micro-injection of NE Evoked Eating

A total of 29 diencephalic sites in 15 animals was tested for NE-elicited eating prior to an injection of 6-OHDA. A site was considered active if 2 g of food or more were consumed within 15 min on two successive occasions after 5 μg of NE were micro-injected in a volume of 0.5 μl. In the seven rats in which 10 active NE sites were found, 6-OHDA

was injected unilaterally. The following day, 5 μg of NE in 0.5 μl were injected at both the 6-OHDA-treated and the nontreated LH sites separated by a one-half to one hour interval. The injections of 5 μg of NE were repeated on the next day so that 6-OHDA's effect on NE-elicited eating could be recorded. A second application of 6-OHDA was performed one week later, but at the contralateral site. This second administration of the drug was followed by another series of NE injections on the ensuing two days. After another one week interval, 8 μg of 6-OHDA was reinjected at the second set of sites so that a total of three injections was completed in each animal. Once again, 5 μg of NE were injected on the two subsequent days.

The 8 animals that did not eat following a micro-injection of NE were subdivided into two groups of 4 animals each. Each animal in one subgroup was given 8 μg 6-OHDA unilaterally in an 0.8 μl volume, and each rat in the other group was given bilateral injections of the compound to determine whether the bilateral injections had more of an effect than the unilateral dose.

The food and water intakes of each animal were monitored for at least one hour subsequent to each injection. Total daily intakes were recorded for as long as 100 days after an injection.

Anatomy

The position of each injection site was verified histologically by injection of 0.5 μl of India ink at the locus according to the procedures described previously [24]. Each rat was anesthetized with nembutal and the brain was perfused via the ascending aorta with a 0.9% physiological saline solution, followed by 10% buffered neutral Formalin. After each brain was sectioned at 30 μ on a Lipshaw freezing microtome, the histological sections were mounted and stained for cells and fibers following a method modified from Klüver and Barrera [17].

RESULTS

When given directly into the hypothalamus, 6-OHDA had a profound effect on eating behavior when injected in a volume smaller than previously used. When injected in a volume ranging from 0.5 to 1.5 μl, 6-OHDA caused not only a marked decline in volitional food and water intakes, but also a loss in body weight.

Behavioral Effects of 6-OHDA Micro-injections

Sleep was the most frequently observed response immediately following the injection of 6-OHDA. In some animals there was instead a brief period of hyperactivity characterized by exploratory behavior. However, following 50 out of 54 injections, the animal appeared to be asleep within 30 min after bilateral or unilateral injections of 6-OHDA.

Long-term changes observed in most of the animals that were adversely affected by the drug were lassitude and what could be described as catalepsy. The catalepsy or some diminished aspect of the symptoms was observed from 2–4 weeks in some animals, and was accompanied by apparent muscular weakness and atonia together with a lack of activity. Each of the animals that never recovered and finally succumbed displayed these symptoms.

Unlike the feeding repsonse reported by Evetts *et al.* [13], 6-OHDA failed to evoke spontaneous eating or drinking in 41 out of 42 rats subsequent to its injection

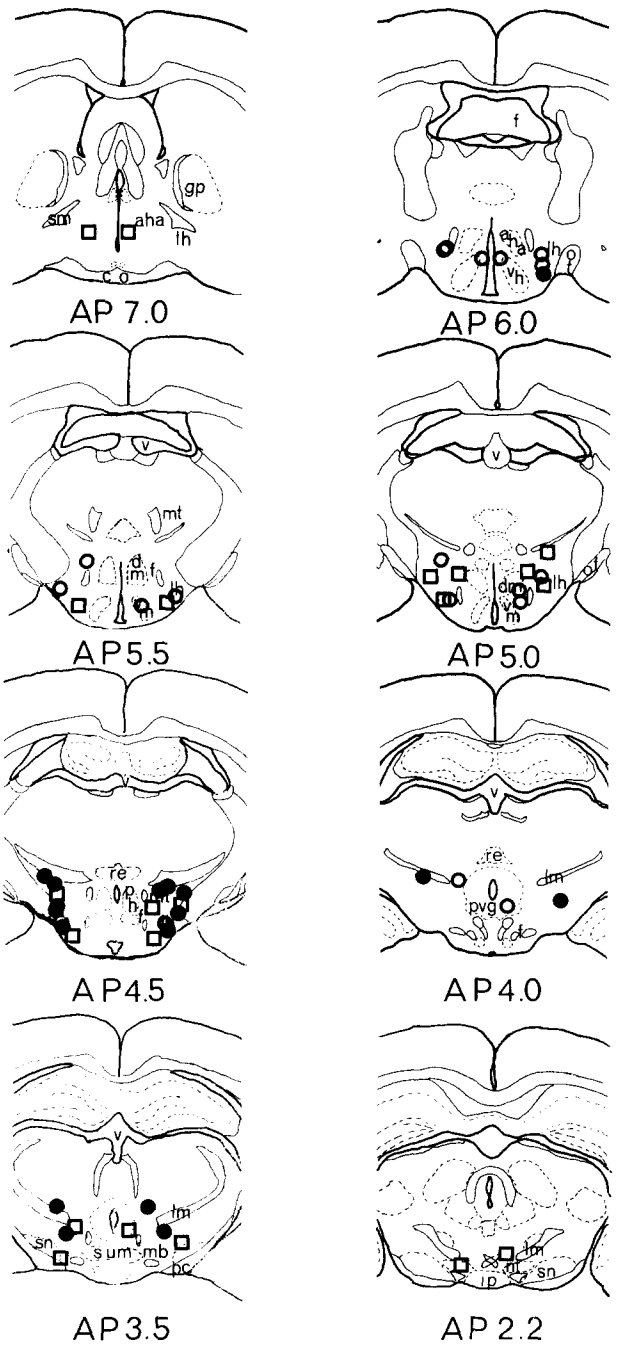

FIG. 1. Morphological mapping within 8 coronal sections of the sites in the brain of the rat at which 6-OHDA in volumes ranging from 0.5 to 1.5 μl was injected and caused: no effect on food intake (○), aphagia reversed by palatable food (□), or death (●). Abbreviations are as follows: sm, stria medullaris; ah, anterior hypothalamus; gp, globus pallidus; lh, lateral hypothalamus; vh-vm, ventromedial hypothalamus; ot, optic tract; mt, mammillothalamic tract; dm, dorsomedial hypothalamus; v, ventricle; re, nucleus reuniens; ph, posterior hypothalamus; lm, medial lemniscus; pvg, peri-venticular gray; sn, substantia nigra; mb, medial forebrain bundle; pc, posterior commissure; sum, decussatio supermammillaris; ip, nucleus intrapeduncularis; and, nt, nucleus of Tsai.

directly into hypothalamic or mesencephalic sites. The exception was an animal (Z-30) that consumed 2 g of food with a latency of 4 min following a unilateral injection of 6-OHDA in the anterior hypothalamus very near the third ventricle at AP = 6.0, L = 0.1, HV = −2.9. In fact, 6-OHDA seemed to inhibit eating responses even in animals reduced to 75% of their body weight, since these rats didn't eat when offered food immediately subsequent to 6-OHDA treatment.

An interesting finding, alluded to by Ungerstedt [45], was an elevated intake of water induced by 6-OHDA in 2 of 42 animals. One animal (Z-27) with a mean daily intake of 44 ml, increased its intake by 30 ml on each of the 2 days subsequent to bilateral 6-OHDA injection into the hypothalamus at AP = 6.0, L = 1.9 and 1.4, HV = −3.4 and −3.5. Another animal increased its daily intake by 40 ml per day over a 12 day period 2 weeks after 6-OHDA administration. The sites of injection for the drug were at AP = 7.0, L = 0.1 and 1.0, HV = −1.4 in the fornical region near the third ventricle.

Lethal Aphagia and Adipsia Induced by 6-OHDA

Following the injection of 6-OHDA, 12 out of 42 animals died within 2 to 26 days. The sites at which the drug exerted its lethal effect are depicted in Fig. 1. The occurrence of death was related to the dose of 6-OHDA. After the highest dose, 5 of 10 rats died, whereas at the three lower doses a total of 7 of 32 rats succumbed.

By offering one of the two diets that were more palatable than the standard powdered chow, the aphagia and concurrent adipsia could be attenuated or reversed. As shown in Fig. 2, an individual rat could either maintain its body weight at a lowered level, or gain weight as long as a palatable food was available. However, as soon as this diet was withdrawn, the animal's food intake declined, body weight dropped, and the animal eventually succumbed.

The severity of the effects of 6-OHDA was not equal in all animals. In fact, 14 of the animals showed only a slight deficit in ingestive behavior in response to bilateral injections of 6-OHDA. Figure 3 illustrates such a result in a rat,

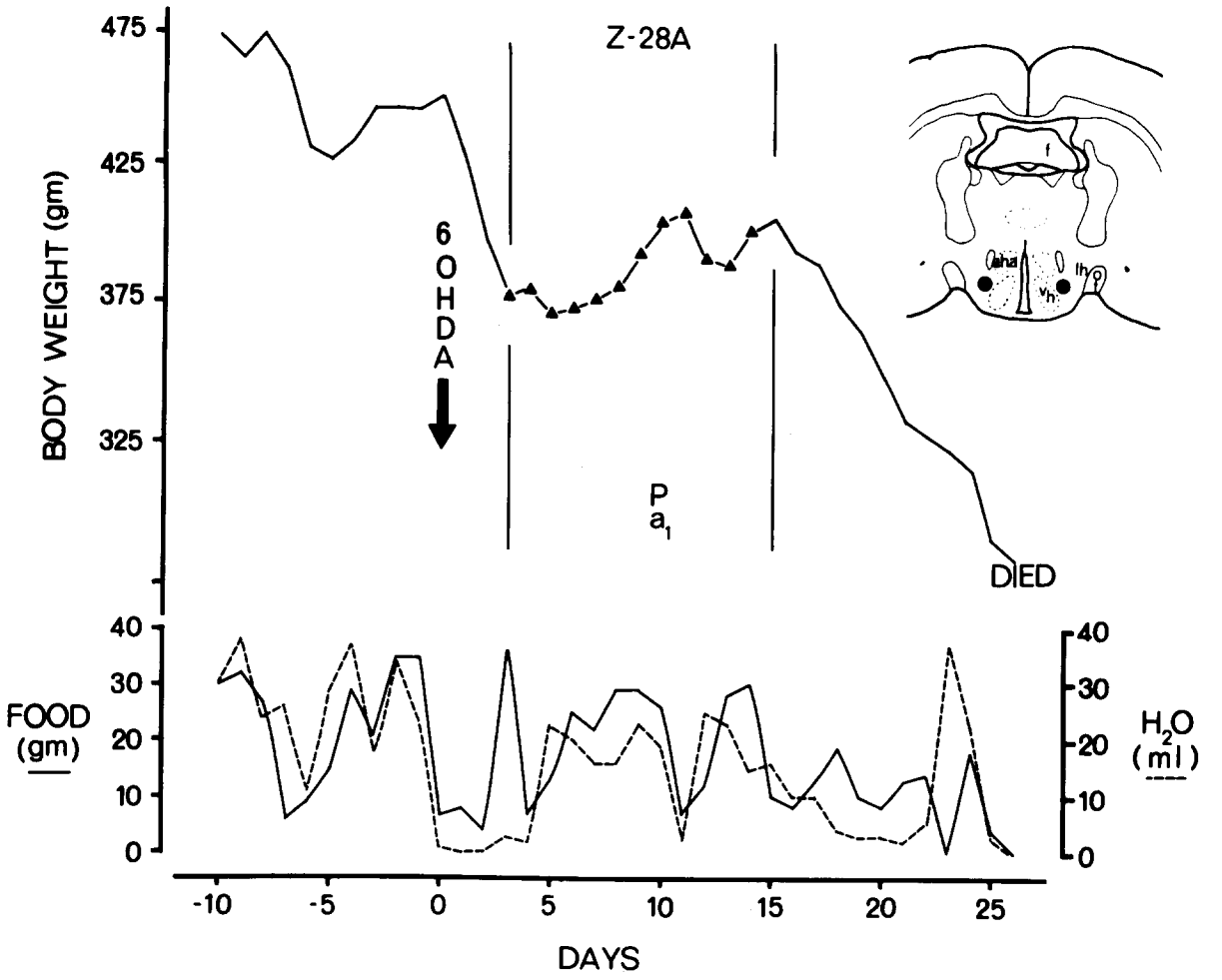

FIG. 2. The body weight (upper) and food and water consumption (lower) recorded daily for Animal Z-28A. 6-OHDA in a dose of 8 μg in 0.8 μl was injected at the site depicted in the coronal section. The triangles (▲) indicate the days on which the enriched palatable diet (Pa_1) was available to the animal ad lib. Powdered food was available on all other days. The arrow (↓) indicates the day on which 6-OHDA was given.

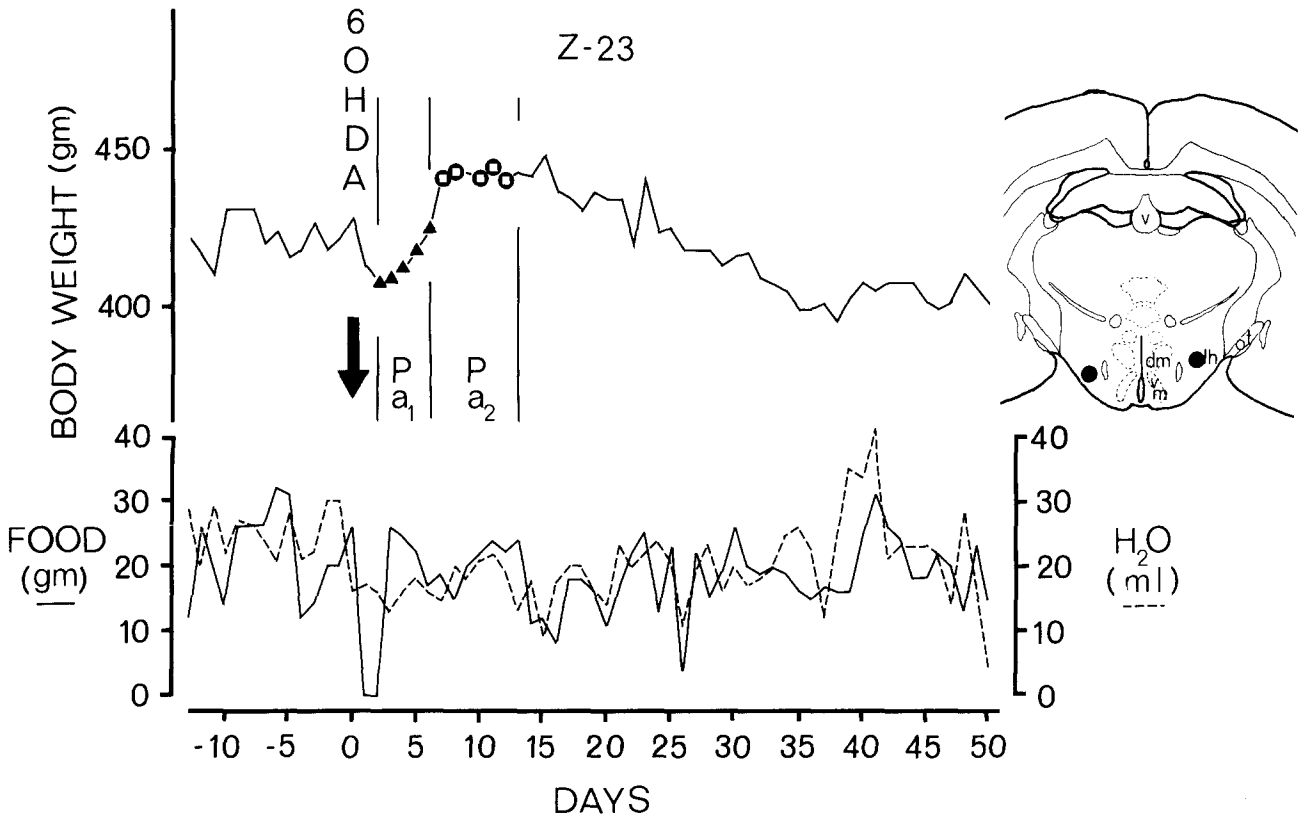

FIG. 3. The body weight (upper), food and water intakes (lower) recorded daily for Animal Z-23 before and following injection of 6-OHDA (↓), 15 μg in 1.5 μl, at the site depicted in the anatomical map. The enriched palatable diet (Pa_1) was offered on days indicated with a triangle (▲), the solid lab blox diet (Pa_2) on days denoted with a circle (○), while powdered food was offered ad lib on all other days.

Z-23, in which 6-OHDA was given at two different sites within the lateral hypothalamus at AP = 5.0, L = 2.0. The animal failed to eat and lost weight for two days, but body weight returned within 5 days to its original level while the rat consumed the enriched diet (Pa_1); the rat maintained the elevated weight when offered the pelletized food (Pa_2). When either of the palatable diets was replaced by the standard powdered chow, the body weight of the rat declined to its normal level once again.

Reversal of 6-OHDA Aphagia and Adipsia

Although 14 rats recovered from the 6-OHDA injection by consuming the normal powdered chow, 28 rats failed to eat and drink appreciably following micro-injections of 5, 8, 15 or 30 μg of 6-OHDA. Unless one or the other of the two palatable diets was offered, the precipitous loss in body weight continued. This critical dependency upon the palatability of the food substance is presented in Fig. 4. Following the bilateral injection of 15 μg 6-OHDA at AP 5.5, the animal failed to eat its normal ration of food and lost considerable weight during the following 3 days. When the palatable enriched chow (Pa_1) was offered on the fourth day, this food was consumed immediately in sufficient quantity that body weight attained its normal level within 5 days. Thereafter, the substitution of the enriched food with the second palatable diet (Pa_2) sustained the recovery of eating during Days 8 through 14 after the 6-OHDA injection. From that day on, the standard chow was consumed and normal intake of water persisted so that the rat regulated its weight and continued to grow. This pattern of recovery was observed in 9 of the 22 animals given palatable diets and was often manifest in different ways. As shown in Fig. 5, offering the rat the enriched diet (Pa_1) halted the sharp decline in the animal's weight caused by the total aphagia and adipsia. However, after the more palatable pelletized diet (Pa_2) was offered, the animal showed by comparison only a slight gain in weight, and again lost weight when the standard powdered chow was offered until 17 days after 6-OHDA injection. Even though the recovery of body weight was sluggish due to the reduced intake of only 5–7 g per day, the animal nevertheless regained a sustaining pattern of eating and drinking 18 days after 6-OHDA was given.

Cyclicity of Palatability-Dependent Food Intake

As illustrated in Figs. 4 and 5, the ostensible finickiness induced by 6-OHDA in some animals had declined approximately 15 to 18 days after the micro-injection. However, it was appartent that in other animals the esculent property of the food was an important factor for periods of up to 90 days. One such animal, Z-19 in Fig. 6, demonstrated clearly the effect of diet on its feeding pattern after administration of 6-OHDA in a dose of 15 μg bilaterally in 1.5 μl at AP 3.5 at the tip of the medial lemniscus. Three days after the

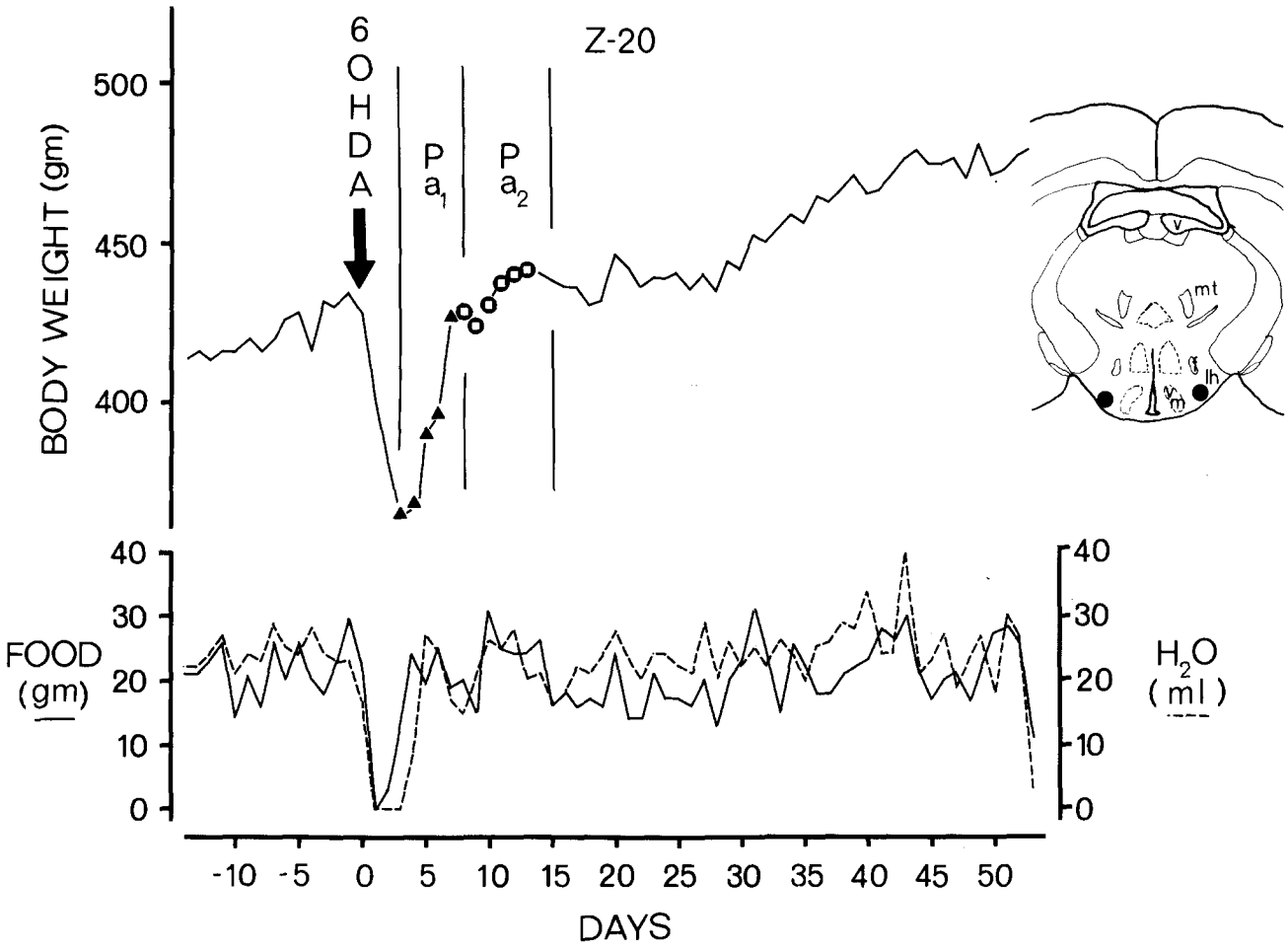

FIG. 4. Daily body weight (upper) and food and water intakes (lower) recorded for Animal Z-20 prior to and following injection of 6-OHDA (15 μg in 1.5 μl) at the site depicted in the coronal section. The more palatable diet (Pa_1) was offered on days denoted with a triangle (▲), the palatable lab blox (Pa_2) on days indicated by a circle (○). On all other days powdered chow was available.

typical eating deficit was produced by 6-OHDA, the animal was offered the enriched diet (Pa_1). As portrayed in Fig. 7, it began to eat and regain its normal body weight, and continued to do so when offered the pelletized diet. However, the animal failed to consume a sufficient portion of food when reoffered the standard lab chow. Rather, it lost weight precipitously, thereby necessitating the reinstatement of the palatable diet (Pa_2). Over the next 40 days, this animal continued to demonstrate this cyclicity in food intake and weight gain. The animal had not yet recovered the ability to maintain its weight on normal lab chow at the time it was sacrificed, 89 days after the bilateral micro-injections of 6-OHDA.

The longevity of the 6-OHDA deficit in ingestive behavior, even after the rat had reached its original body weight, is demonstrated in Fig. 7. Following two successive intervals of 5 and 2 days respectively, in which the enriched palatable diet (Pa_1) was consumed, the pelletized diet (Pa_2) was offered from days 38 through 53 after the 6-OHDA injections. During this latter 16 day period, the rat consumed food and water in amounts greater than normal and gained well over 100 g. However, when the regimen of powdered lab chow was reinstated, the rat suddenly became hypophagic and hypodipsic again, and lost body weight concomitantly until the pelletized diet was again offered on Day 63.

6-OHDA and the Set-Point for Body Weight Regulation

When reduced to 75% of normal body weight prior to receiving a hypothalamic injection of 6-OHDA, 4 of 13 rats died independent of the dose given. One of four died at the low dose of 6-OHDA (5 μg), two of four at the medium dose (15 μg), and one of five at the high dose (30 μg). This is in contrast to an apparent relationship between the high dose of 6-OHDA and death observed for the nondeprived group, in that four of five animals at normal body weight succumbed to the high dose of the drug, none died following the medium dose and only one after the low dose. As shown in Fig. 8, evidence was obtained for an alteration of set-point for body weight in one of the deprived animals. Aphagia together with adipsia and weight loss followed 6-OHDA treatment for two days only. However, contrary to the other animals' behavior, the rat, although refusing to

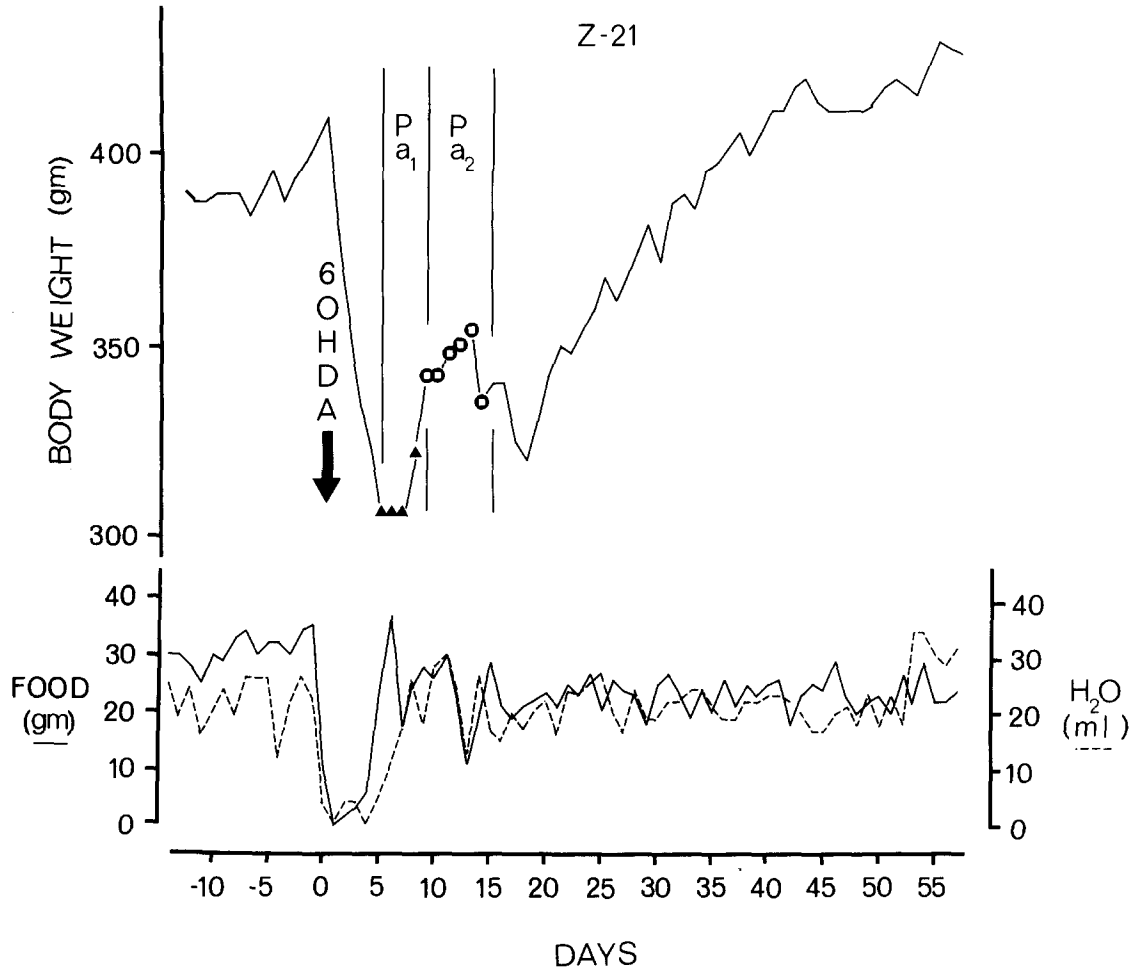

FIG. 5. Daily body weight (upper) and food and water intakes (lower) recorded for Animal Z-21 prior and subsequent to injection of 6-OHDA (15 μg in 1.5 μl) at AP = 5.4, L = 1.5, HV = −3.7 [11]. Histology could not be obtained for this animal. The triangles (▲) and circles (○) indicate the days on which the palatable diets were offered. Powdered food was available on all other days.

eat for the initial 2 days after 6-OHDA, became surprisingly hyperphagic on the third and fourth days. The animal ingested the powdered lab diet in quantities of 33 and 29 g respectively per day, and than became hypophagic for the next five days. Seemingly, by the tenth day after 6-OHDA treatment, the animal had recovered its normal ingestive pattern and began to regain its body weight. In conjunction with a possible altered set-point, the animal had not regained its pre-injection weight even 100 days after the injection of 6-OHDA; instead the weight remained at about 90% of its predeprivation weight.

The results recorded for Rat Z-12 illustrate the typical response for those rats in which the reduction in body weight seemed to facilitate the recovery from the 6-OHDA injection. Figure 9 shows that the animal displayed anorexia for 2 days after 6-OHDA was injected, but subsequently began to eat the pelletized diet. The time required to attain the normal weight level was approximately 30 days. In 6 of 9 cases, the deprived animal that did recover from 6-OHDA typically regained its former weight level within 20 to 50 days. A comparison of Fig. 9 with Figs. 4 and 5 reveals that the recovery from an injection of 6-OHDA into the hypothalamus occurs at a similar rate for both normal animals and those deprived to 75 percent of their previous body weight. Food intake and weight gain were facilitated by either of the specially prepared diets (Pa_1 or Pa_2) for an initial period after the drug was administered; generally, the animals could thereafter maintain normal ingestive behavior when offered powdered chow.

Other evidence of a lowered set-point for body weight regulation produced by 6-OHDA was provided in several animals that were not food-deprived prior to the drug injection. As shown in Fig. 10, a loss of both appetite and body weight was the initial response for 3 days following a unilateral injection of 8 μg of 6-OHDA at the level of AP 3.5 and at the depth shown in the anatomical map (inset). The animal continued to lose weight even after the enriched diet (Pa_1) was offered on Day 4 after 6-OHDA. However, even after a significant amount of this palatable food was consumed for the eleven days that it was offered, the rat's weight remained at a level that was 25–30 g below its baseline weight. After the standard powdered food was offered on the 14th day after 6-OHDA, the body weight of

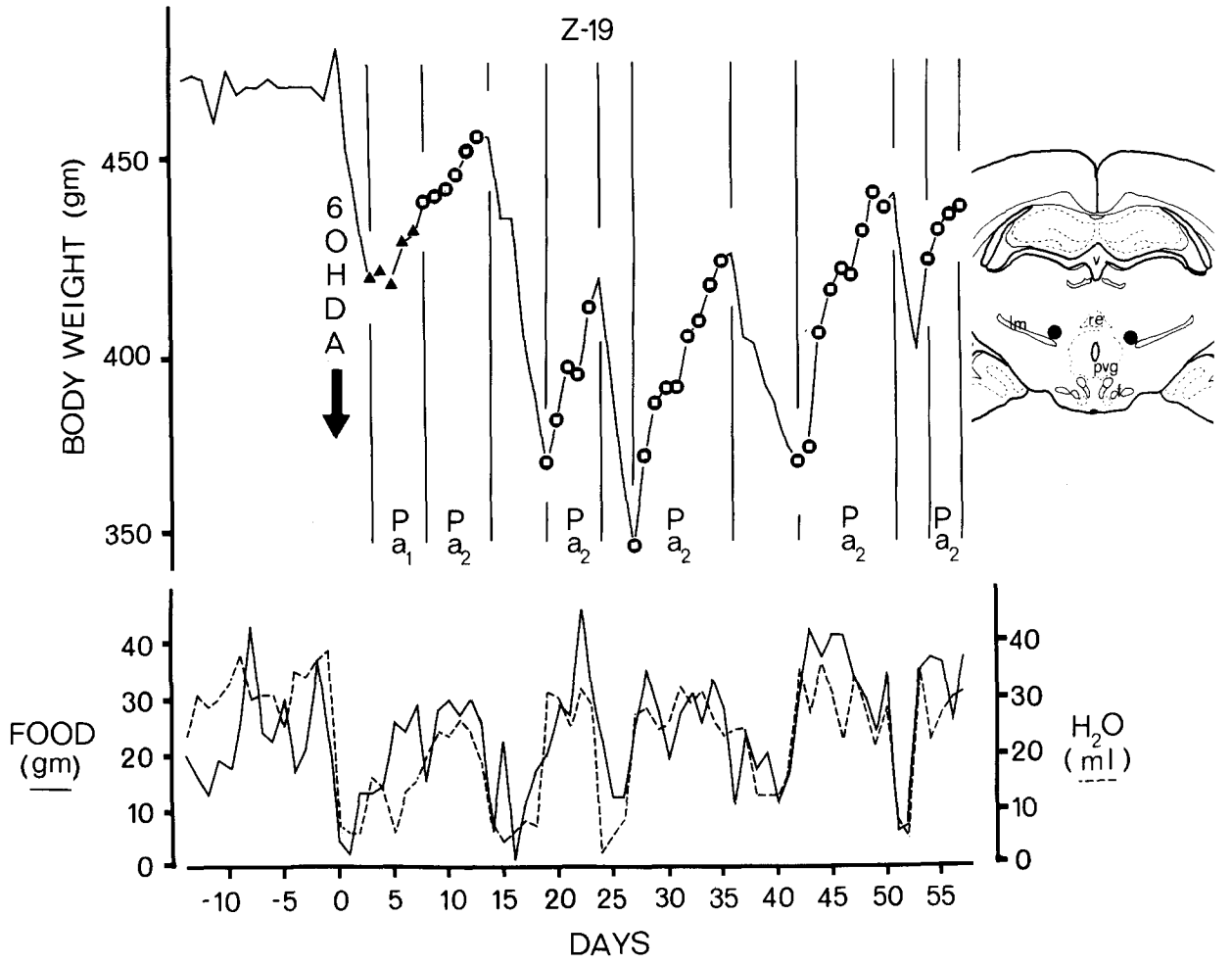

FIG. 6. Body weight (upper) and food and water intake (lower) recorded daily for Animal Z-19 prior and subsequent to injection of 6-OHDA (↓) (15 μg in 1.5 μl) at the site indicated in the anatomical map. The triangles (▲) indicate the days on which the enriched palatable diet (Pa_1) was available to the animal ad lib. The solid lab blox diet (Pa_2) was available on days denoted with a circle (○), while powdered food was available on all other days.

the rat declined steadily over the next 30 day period to approximately 80 percent of its normal body weight. The hypophagia and loss in body weight were reversed by offering the pelleted diet for eight days, but an upward trend in body weight toward the pre-6-OHDA level was not observed.

6-OHDA and Norepinephrine Induced Eating

Norepinephrine was injected in a dose and volume indicated in Table 1 after the injection of 6-OHDA in an attempt to elicit eating, both at sites which were pre-tested for NE elicited eating and at sites at which no NE pretest had been conducted. The results of these injections are depicted in Fig. 11. At 12 brain sites, depicted by the triangles (▲), an injection of NE prior to 6-OHDA treatment failed to elicit an eating response. Prior to the injection of 6-OHDA, eating was elicited at 10 sites in 7 animals following an injection of NE. However, after an injection of 8 μg of 6-OHDA in 0.8 μl to these same sites, an evoked eating response was observed following application of NE at only 5 of these 10 sites when they were retested. The sites which remained active to NE, both before and after 6-OHDA, are indicated by the closed square (■). Two such sites were almost coincident in the basal hypothalamus at AP 6.0. The 5 sites at which NE no longer was effective in inducing ingestive behavior are depicted by the open squares (□).

Although no pre-6-OHDA screening for sensitivity to NE was conducted at many sites, 34 sites in the brain located in regions from which eating is known to be evoked by NE [4, 5, 10, 15, 33] were tested for NE eating following 6-OHDA. At 32 of these sites, no eating was observed subsequent to the norepinephrine administration, and eating occurred following a 10 μg injection at only two sites.

In addition to norepinephrine, dopamine (6 sites), angiotensin (10 sites), and the eserine-acetylcholine mixture (6 sites) failed to elicit either feeding or drinking when they were injected at the sites depicted in Fig. 12. Further, calcium injected into the ventricle was not effective in inducing any food consumption in three animals tested after 6-OHDA.

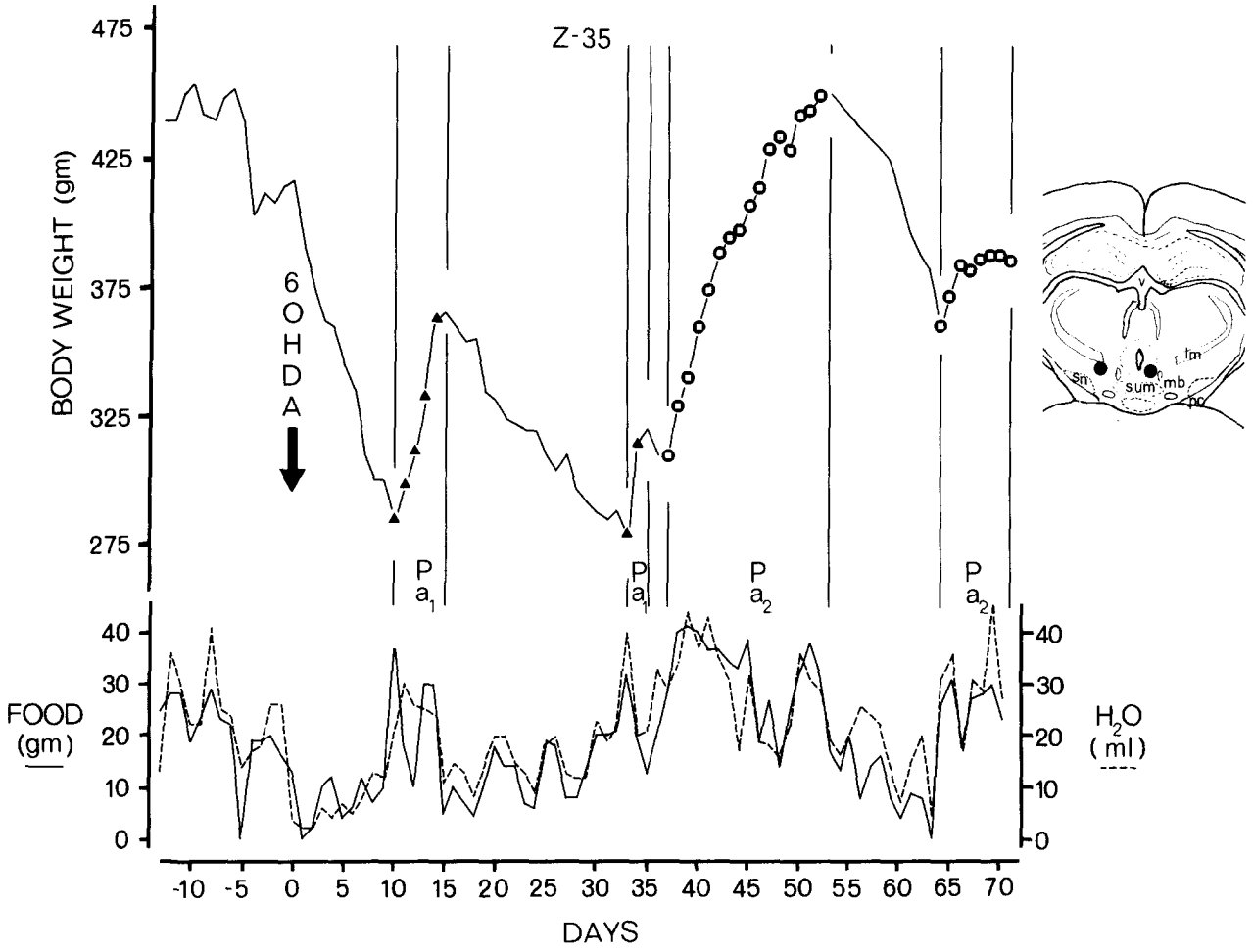

FIG. 7. Body weight (upper) and food and water intake (lower) recorded daily for Animal Z-35 prior and subsequent to injection of 6-OHDA (8 μg in 0.8 μl) at the cerebral site indicated in the coronal section. Powdered food was available at all times except when either of the palatable diets, Pa_1 (▲) or Pa_2 (○), were offered in its place.

Anatomy

Representative histological sections upon which the composite mapping of the injection sites presented in Figs. 1, 11 and 12 was based are represented in Fig. 13 for 4 animals. In A (upper left), sites close to the lateral hypothalamus at AP 6.0 are shown at which the injection of NE elicited an eating response at the site on the left. Following an injection of 8 μg of 6-OHDA in a volume of 0.8 μl, this NE-induced eating response was abolished. In the animal depicted in B (upper right), 15 μg of 6-OHDA was given bilaterally in 1.5 μl at sites located in the substantia nigra and at the tip of the medial lemniscus, after which the rat became aphagic and lost weight for 2 days (Fig. 9), then consumed palatable food, and eventually regained its normal body weight. The rat depicted in C (lower left) was given 5 μg of NE at each site prior to a unilateral injection of 8 μg of 6-OHDA in 0.8 μl at the right side. Injection of NE did not produce an eating response, but 6-OHDA was followed by a long-term diminution in body weight (Fig. 11) when it was injected into the brainstem of the rat at AP 2.2 just lateral to the decussation of Forel. In D (lower right), sites at AP 3.5 in the periaqueductal gray and at the tip of the medial lemniscus, 5 μg of NE did not induce an eating response at either site when injected in a volume of 0.5 μl. When 8 μg of 6-OHDA was injected bilaterally, the animal's food intake became dependent on the sapidity of its diet (Fig. 7).

DISCUSSION

The perturbation of ingestive behavior following the hypothalamic injection of 6-OHDA in some respects is comparable to that following an electrolytic lesion in a corresponding anatomical region. However, the abnormality in food consumption, noted after the chemical lesion of the hypothalamus differs in several ways from those produced by an electrolytic lesion.

First, even though gastric intubation of food was not employed in this experiment, only 12 of 42 animals died following the treatment with 6-OHDA. Hence, the chemical lesion, more localized by virtue of the 0.5–1.5 μl volume than in other investigations [34,45], does not appear to be as severe as that following the circumscribed destruction of

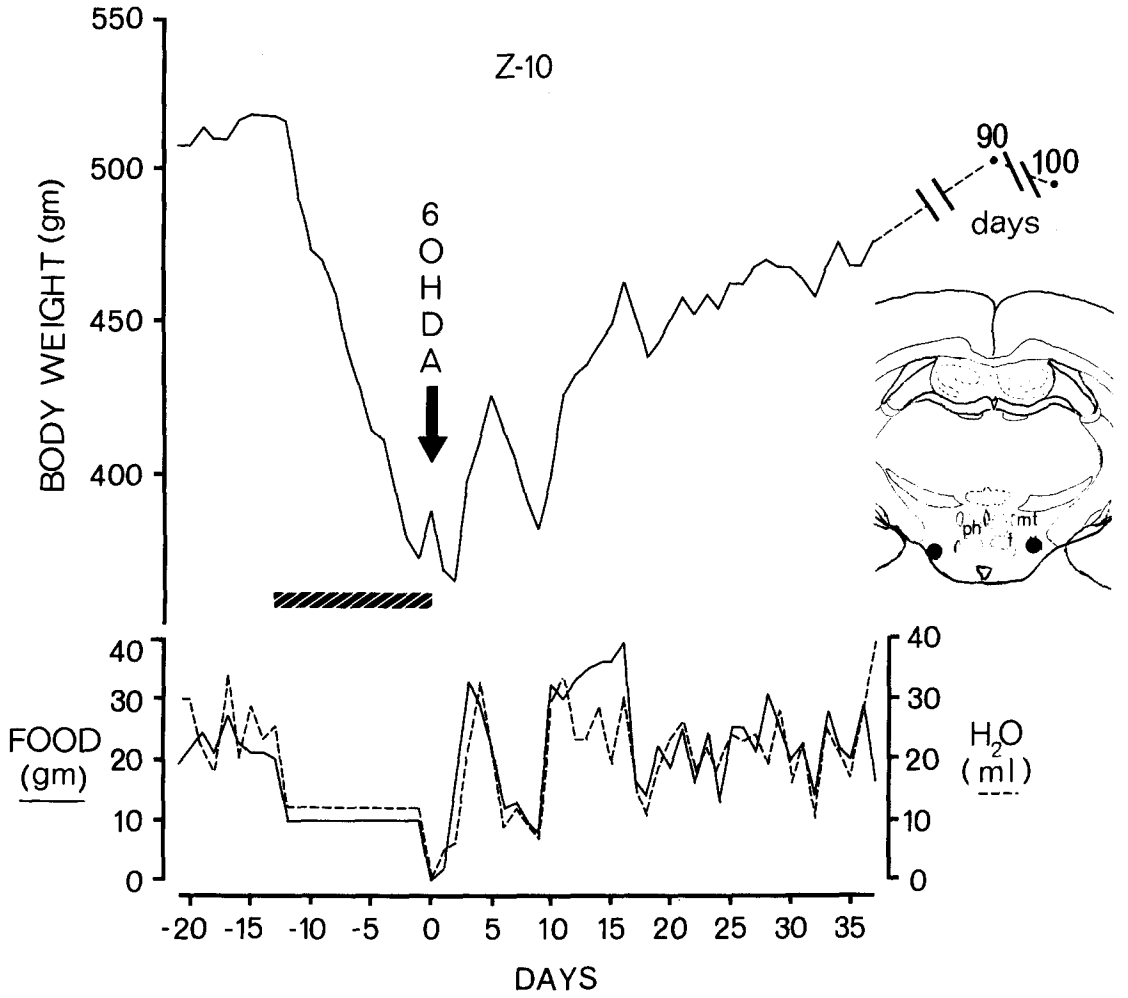

FIG. 8. Body weight (upper) and food and water intakes (lower) following bilateral injections of 6-OHDA (15 μg in 0.75 μl) at the cerebral sites indicated in the coronal map. The striped bar indicates the period of deprivation prior to 6-OHDA administration. Other than during deprivation, powdered food was available ad lib throughout the experiment.

the corresponding area by an electrolytic lesion. As indicated by Teitelbaum and Epstein [38] the aphagia and adipsia produced by the latter method ordinarily is reversible only after intragastric feeding.

Second, Stage II of the classical lateral hypothalamic syndrome in which the animal becomes anorexic and remains adipsic [38] was not observed in the present experiments. A rat in Stage II eats appreciable quantities of palatable food but not enough to maintain body weight and it dies if not tube fed. Instead, following the injection of 6-OHDA, the rat either refused food and water altogether or consumed a sufficient amount of palatable food and water so that body weight and adequate hydration were maintained.

Third, the exaggerated role of palatability or taste sensitivity is well documented in the pattern of ingestive behavior that develops after a diencephalic lesion [9, 22, 36, 37, 38, 40], in that electrolytic destruction of the lateral hypothalamus apparently accentuates the sensitivity of the rat to a negative tasting substance [38]. On the other hand, 6-OHDA seems to cause the rat to reject its normal food, but then to consume highly palatable chow. Although the factor of palatability of the food was not considered in earlier studies on the effects of 6-OHDA injected in a large volume into subcortical structures [34,45], this single factor may explain why in those investigations such a high percentage of 6-OHDA-treated rats succumbed.

Fourth, an electrolytic lesion of the lateral hypothalamus can impair drinking even more severely than feeding behavior [38]. However, after 6-OHDA was micro-injected, sufficient prandial drinking did occur to maintain each rat in water balance. This result differs from a previous study concerning the effects of 6-OHDA on fluid intake [34], but is in agreement with a report of the effects on food intake of 6-hydroxydopa administered peripherally [39] and another study that examined the effects of intraventricular 6-OHDA on drinking [35].

An equally crucial point to be considered is that 6-OHDA interfered with the normal food consumption even at the low (5 μg) dose. This result correlates well with the findings of histochemcial fluorescence that an intracranial dose as small as 0.2 μg can deplete endogenous

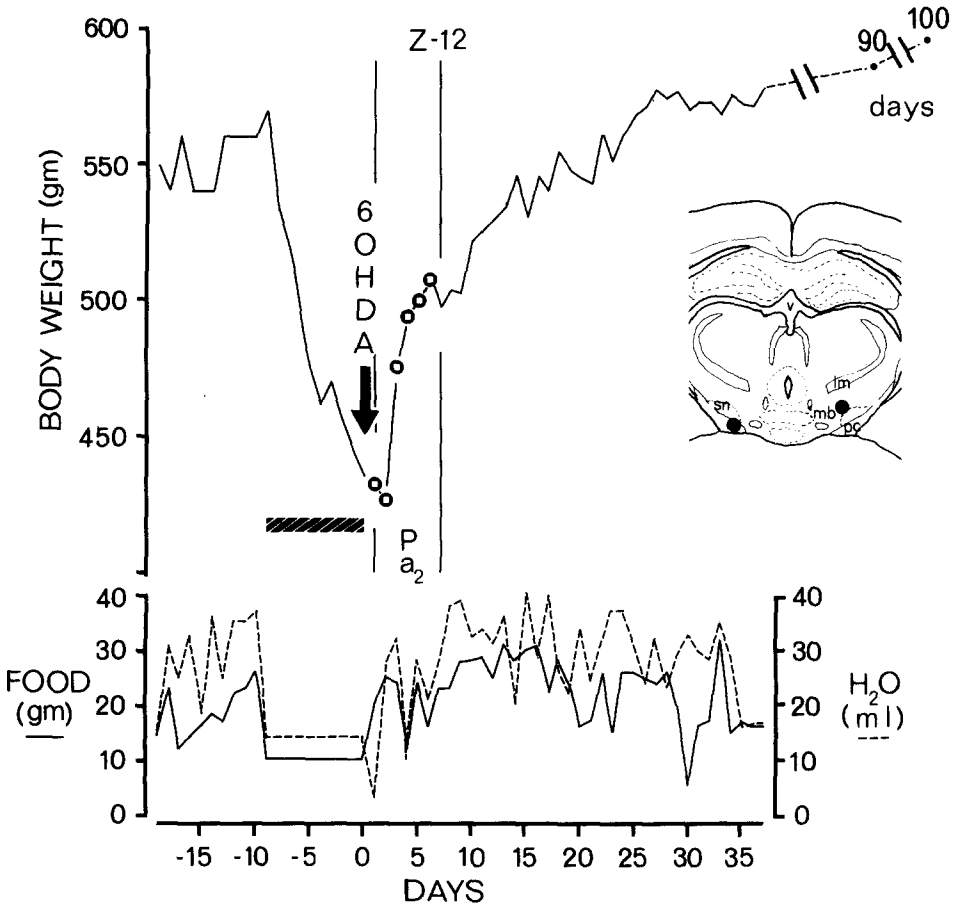

FIG. 9. Body weight (upper) and food and water intakes (lower) recorded daily for Animal Z-12 prior to and following injection of 6-OHDA (15 μg in 0.75 μl) at the sites depicted in the coronal section. The palatable pelletized diet was offered on days denoted by a circle (○). The striped bar indicates the period of deprivation during which the animal's food intake was limited to 10 gm per day. Ad lib lab chow was available at all other times.

catecholamines [46]. It has recently been reported that the injection of 6-OHDA into only one lateral ventricle of the brain causes asymmetric damage to brain catecholamine structures [48]. Hence, it is possible that the absence of behavioral deficits after ventricular injections [3, 32, 47] may be due to the failure to inactivate bilaterally vital catecholaminergic pathways. Therefore, it is essential that the interaction between the various ascending and descending systems is examined in this species using a direct micro-injection into the brain. Recently, it has been shown that bilateral injections of 6-OHDA into the cerebral ventricles in repeated doses do affect food intake of the rat [51].

The fact that each of the four doses of the drug could produce a lethal aphagia-adipsia in the rat suggests that some of the injected 6-OHDA destroyed a principal aminergic pathway which subserves the ingestive function. On the other hand, several injections affected key cells to a lesser degree which enabled the palatability factor to reverse the aphagic syndrome. In any event, anatomical localization of the sites of action of 6-OHDA not available prior to this time can be determined from our data. When 6-OHDA was injected into the LH at the tip of the internal capsule, it almost always had an adverse effect on food intake. In the plane of AP 4.5 in the DeGroot atlas, norepinephrine cell bodies as well as NE and dopamine cell fibers are present at the sites of injection [43]; it is not possible to estimate which if either of these two amines are principally affected. At the level of the substantia nigra, the two sets of lesions which produced fatalities were intermingled among NE and DA axons. Hence, once again it is not possible to specify whether a dopamine or norepinephrine system is primarily involved in the chemical lesion and the detrimental action on food intake. Although the volume of the injections used in this experiment minimizes the extent of diffusion, it still is difficult to distinguish the dopamine from norepinephrine sites that were affected.

With regard to the Powley-Keesey theory, evidence was obtained that lowering the body weight of the rat prior to 6-OHDA administration did not necessarily ameliorate the disruption of food intake with but one exception in which the largest dose was given. For the most part, the deprived

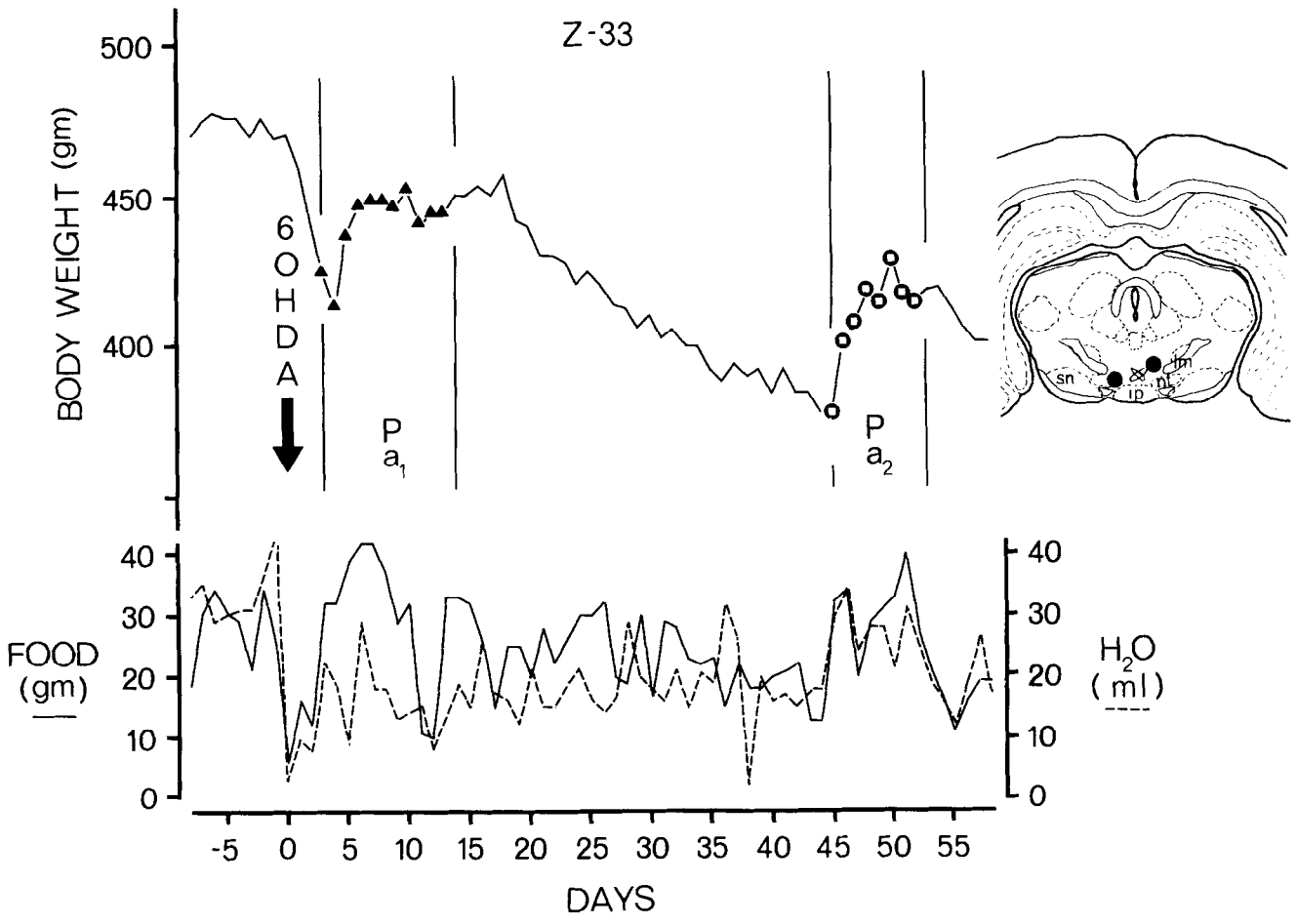

FIG. 10. Body weight (upper) and food and water intakes (lower) recorded daily for Animal Z-33 prior and subsequent to injection of 6-OHDA (8 μg in 0.8 μl) at the cerebral site on the right side. Palatable food was available on days denoted by a triangle (▲) (Pa_1) or by a circle (○) (Pa_2), as in the previous figures, and powdered food was available on all other days.

animal either succumbed as a result of the lesion or regained its former body weight by consuming palatable chow. Neither outcome was predicted by the set-point theory of Powley-Keesey [28]. However, these findings are not conclusive evidence against the existence of a hypothalamic set-point, but indicate that a catecholamine may not be the only neurohumoral factor involved in controlling such a system [25]. Mufson and Wampler [22] suggested that increased sensitivity to the taste of the food caused a lowering of body weight seen in an animal with an electrolytic LH lesion, rather than the resetting of an internal set-point mechanism for body weight. The present experiments indicate that a similar mechanism could be operative after a discrete chemical lesion within the diencephalon. For example, only one animal deprived to 75% of its normal weight reset its body weight to a lower level subsequent to 6-OHDA administration. Of the 12 other deprived animals, four succumbed. The remaining eight rats consumed food normally and regained or surpassed their former weight. Further, once treated with 6-OHDA, the animals that had not been deprived lost weight when given normal food, and maintained or gained weight only when given palatable chow. This, too, could be interpreted as an alteration of the rat's sensitivity to the taste of its food.

In support of the set-point theory of Powley and Keesey, a greater percentage of animals survived the high dose (30 μg) of 6-OHDA when deprived, indicating that the animal's preinjection body weight may alter the effect of the chemical lesion of the hypothalamus. In addition, one animal (Z-33 in Fig. 10) provided strong evidence for an altered set-point. Although consuming near normal amounts of dry chow, it steadily lost weight, indicating that taste alone was not the predisposing factor in the decline in body weight. Another animal (Z-10 in Fig. 8) also displayed the major components of the behavior of the deprived animal according to the Powley-Keesey theory. First, it displayed a hyperphagia after a 6-OHDA lesion; second, it did not regain its normal body weight. Taken together, it is clear from the variations in these responses that a bio-

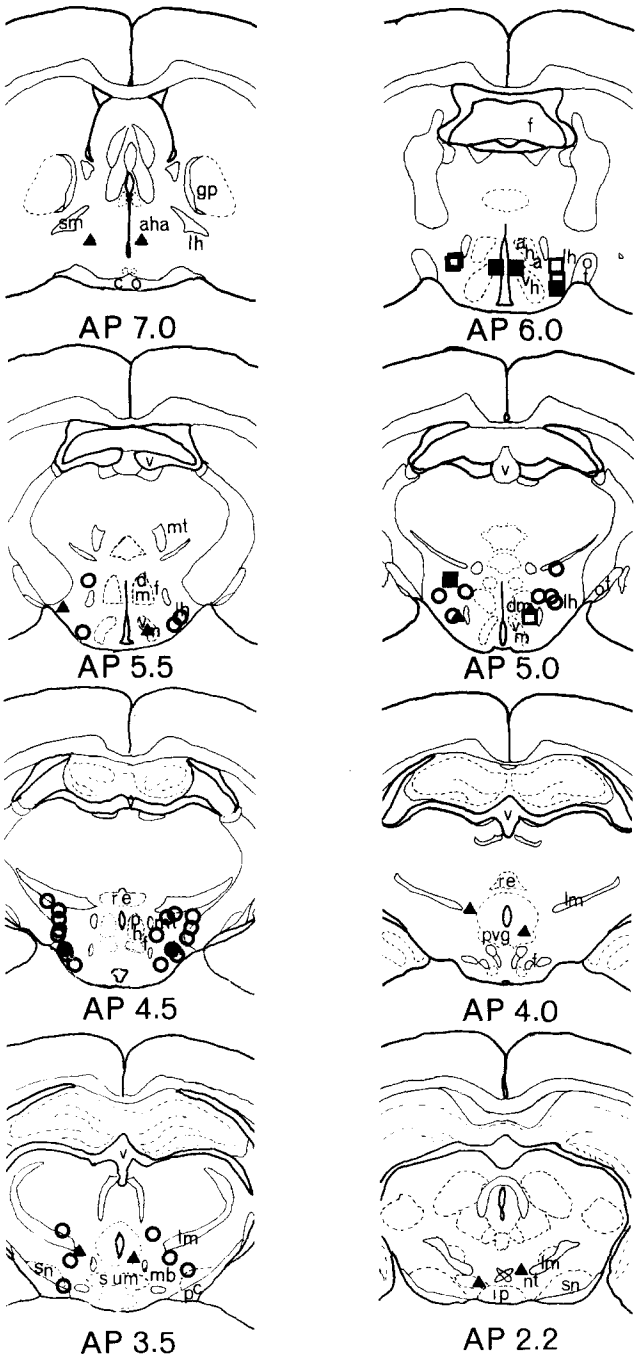

FIG. 11. A morphological mapping within eight coronal sections of the 57 sites in the brain of the rat at which norepinephrine was injected in an attempt to elicit eating. Areas indicated by a triangle (▲) indicate a site at which NE did not elicit eating prior to 6-OHDA. An anatomical region depicted by a square is a site at which NE-elicited eating was reliably observed prior to 6-OHDA administration: the solid square (■) indicates a site that remained sensitive to NE after 6-OHDA, whereas an open square (□) indicates a site that was no longer active. A circle depicts a site at which NE was not injected prior to 6-OHDA administration; an open circle (○) indicates a site at which NE did not elicit eating subsequent to 6-OHDA whereas at the two sites indicated by the black circle (●), a 10 μg injection of NE did evoke eating after 6-OHDA.

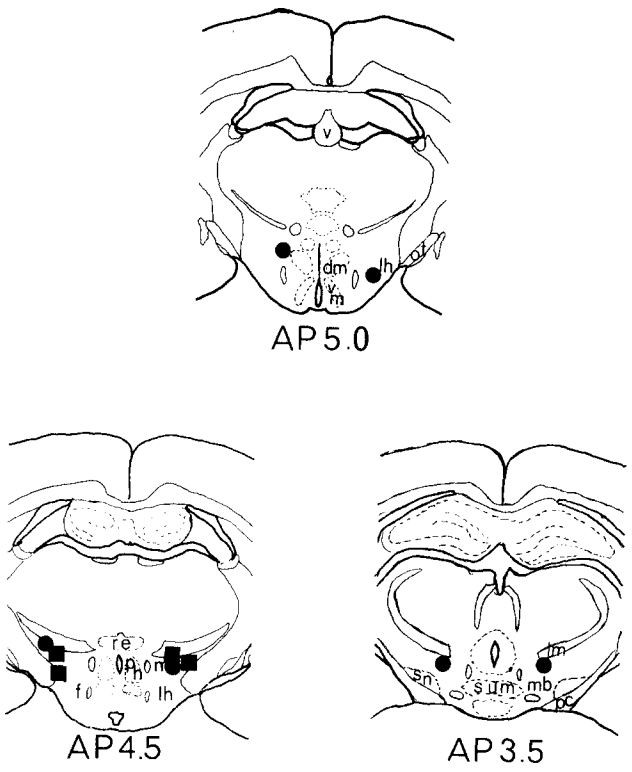

FIG. 12. Sites in the rat at which a micro-injection of one of the following compounds failed to elicit an eating response: acetylcholine-eserine, dopamine or angiotensin. The regions indicated with a black square (■) were not sensitive to angiotensin or dopamine, while those indicated with a filled circle (●) were nonresponsive to angiotensin, dopamine or acetylcholine-eserine.

chemical lesion caused by 6-OHDA may or may not alter a hypothetical set-point for body weight, with such factors as size of lesion, its locus and the esculent nature of the food being critical.

Another interesting observation was that, with the exception of one site, significant feeding was not observed following the micro-injection of NE. In addition, 50% of the brain sites at which NE had induced eating prior to the 6-OHDA micro-injection, were not viable following the injection of the drug. Neither of these results were expected since a stimulus-bound eating response to 6-OHDA and persistent NE evoked eating have been previously reported [13]. Sleep or sedation, as previously observed [20], was the most reliably observed behavioral response subsequent to the treatment with 6-OHDA. Whether the effect is a result of atrophy in the terminal due to endogenous NE depletion, to an overloading of supersensitive synaptic units or to some other unknown factor is yet to be demonstrated. The supersensitivity reported by others [13,44] was not readily observed.

Since neither Ca^{++}, dopamine, angiotensin, nor acetylcholine-eserine could elicit eating when injected at cerebral sites following the injection of 6-OHDA, it is possible that the ingestive behavior produced by these compounds may be mediated at least partially through the release of NE [49,50]. Although the sites at which these

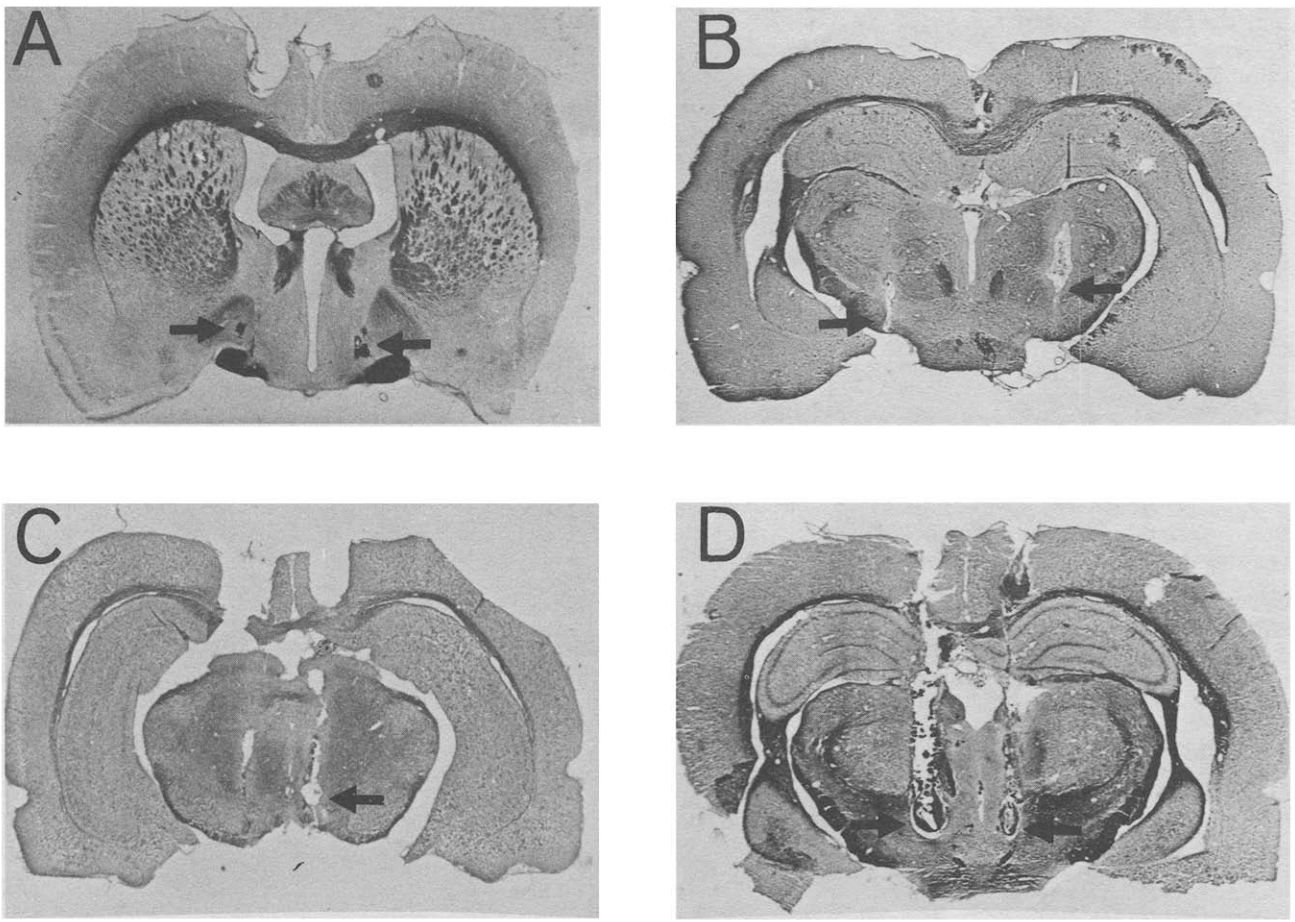

FIG. 13. Histological sections cut in the coronal plane at 30 μ. The arrows indicate the site of 6-OHDA injection. See text for explanation.

compounds were administered were not tested with the drugs before the application of 6-OHDA, nevertheless they are homologous to those at which eating is elicited by these compounds [12, 15, 26]. It does not therefore seem unreasonable to attribute the lack of response to the profound local effect of 6-OHDA. Both Ca^{++} [30] and acetylcholine [8] facilitate the release of NE from storage granules, and angiotensin may inhibit the reuptake of NE [16]. It is possible that the destruction of endogenous stores of NE by 6-OHDA may abolish the eating response induced by these other compounds by removing the pharmacological mechanism through which they mediate their effect. Dopamine injected into the brain elicits eating but after a much longer latency than that of NE [15,26]; this suggests that dopamine may have to be synthesized into NE before it exerts its action on the neurons involved in feeding. Also, 6-hydroxydopamine decreases the synthesis of dopamine-β-hydroxylase, the enzyme which converts NE to DA, both in the periphery [7] and the CNS [29]. Finally, the reason that dopamine failed to elicit eating in the present experiment may be because it was not converted to NE in the absence of dopamine-β-hydroxylase. Evidence for this supposition will be provided by additional research which will further clarify the role of catecholamines in the initiation of feeding behavior.

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